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ANTI-RCC X ANTI-DTPA BISPECIFIC MONOCLONAL ANTIBODIES FOR TWO-PHASE TARGETING OF RENAL-CELL CARCINOMA

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We aim to use bispecific monoclonal antibodies (bsMAbs) for targeting radiolabeled diethylene triamine pentaacetic acid (DTPA) to renal-cell carcinoma (RCC). In these pretargeting protocols the anti-tumor bsMAb is administered and allowed to clear from the circulation whereafter the radionuclide is given as a small rapidly clearing ligand. A pretargeting protocol may optimize radioimmunodetection and radioimmunotherapy of tumors in vivo.

Five anti-DTPA MAbs were produced reactive with DTPA loaded with different metals. This broad reactivity of the MAbs make them suitable for radioimmunodetection (using ^{111}In-DTPA or ^{99m}Tc-DTPA) as well as radioimmunotherapy (with ^{90}Y-DTPA). The ability of the anti-DTPA MAb DTIn1 to bind ^{99m}Tc-DTPA in vivo was tested in rats with focal *S. aureus* infection. Antibodies are known to nonspecifically accumulate in infectious foci. Priming with DTIn1 followed by ^{99m}Tc-DTPA resulted in rapid visualization of the abscess within 4 hr demonstrating that ^{99m}Tc-DTPA was specifically trapped by DTIn1. Priming with an irrelevant control antibody didn't result in visualization of the abscess. The retention of ^{99m}Tc-DTPA in the circulation by DTIn1 could be blocked by injection of a 10-fold molar excess of BSA-DTPA-In 30 minutes prior to injection of ^{99m}Tc-DTPA. These experiments indicate the feasibility of two- as well as three-phase targeting protocols in vivo with this anti-DTPA MAb.

The anti-DTPA MAb producing hybridomas were used for somatic cellfusion with hybridoma G250 directed against RCC, resulting in three bsMAb producing cell lines (G250 x DTIn1, G250 x DTY4 and G250 x DTIn2). Individual purification protocols were developed using protein A affinity chromatography followed by hydroxylapatite chromatography and/or cation exchange chromatography resulting in highly purified functionally active bsMAbs. The affinity constants of the bsMAbs for binding ^{111}In-DTPA were similar to the affinity constants of the parental anti-DTPA MAbs indicating that they can be used in targeting protocols.

Currently the ability of these purified bsMAbs to target radiolabeled DTPA to RCC xenografts is tested in a nude mouse model. In these experiments the tumor is pretargeted with bsMAb, followed by the injection of ^{111}In-DTPA.

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GERM-LINE MUTATION OF VON HIPPEL-LINDAU TUMOR SUPPRESSOR GENE: GENOTYPES ASSOCIATED WITH RENAL CELL CARCINOMA PHENOTYPE.

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Von Hippel-Lindau disease is a familial cancer syndrom demonstrating autosomal dominant inheritance. Renal cell carcinoma is the leading cause of death in this disease. The reported VHL gene cDNA contains an open reading frame of 855 bp together with a 3'-untranslated region. The exact 5' end and initial codon of the coding region remain unknown. Refering to both sequence data of human and mouse VHL genes, we used 6 pairs of primers to cover the reported ORF and splicing sites in the mutation study of our 10 Swedish VHL families. Direct genomic DNA sequencing was used to detect the mutation.

An additional G was found before the first bp of the reported ORF that made up a potential splicing site. We also made clear the consensus sequence flanking the splicing site of each exon. Six mutations(4 were novel) were mapped to exon 1 and 3 of which 2 were single bp deletions and 4 were single base substitutions. Two large germ-line deletions were detected by Southern blot of EcoRI or PstI digestion. Four mutations that caused significant protein changes all predisposed to VHL associated renal clear cell carcinomas. Overall 8 germ-line VHL alterations were detected in 10 unrelated VHL families and additional 5 disease gene carriers were also distinguished.

Our data suggests that the reported ORF have already contained the complete coding sequence of the VHL protein and an additional 5'-untranslated region might exist. Mutation studies of VHL families are of high importance in clinical detection of disease gene carriers. Analysis of genotype and phenotype relation might provide insight in the VHL gene function.

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MOLECULAR CHARACTERIZATION OF THE RENAL CELL CARCINOMA-ASSOCIATED ANTIGEN G250. *Egbert Oosterwijk, Mirjam de Weijert, Adrie van Bokhoven, Ruud H Brakenhoff*, Wilhelmus P Peeters, Frans MJ Debruyne.* Urological Research Laboratory, Academic Hospital Nijmegen, 6500 HB Nijmegen, * Free University Hospital, Amsterdam, The Netherlands.

The Renal Cell Carcinoma (RCC) associated antigen G250 was initially identified by monoclonal antibody G250 (mAbG250). The antigen is homogeneously expressed in >70% of RCCs and is absent in normal kidney and other normal human tissues, with the exception of gastric mucosal cells and cells of the larger bile ducts. Clinical trials with ^{131}I labeled mAbG250 have indicated that the antigen is a potential therapeutic target.

Thus far, the molecular characterization of G250 antigen has shown that the antigen has the characteristics of a protein. Further molecular characterization has been hampered by the apparent extreme sensitivity of the G250 antigen for non-native conditions.

For the molecular cloning of the cDNA encoding the G250 antigen, a eukaryotic expression cloning strategy was used. A cDNA library was constructed using mRNA from a human RCC cell line in the eukaryotic expression vector pCDM8. Immunohistochemical screening of progressively smaller pools of cDNA which were transfected into COS cells has lead to the isolation of a cDNA (pMW1) of approximately 1.5 kb containing an open reading frame and having a polyadenylation signal was isolated. Transfection of this cDNA into a G250 HCC cell line resulted in the expression of G250 immunodetectable protein, showing that this cDNA indeed encodes the G250 antigen. Northern analysis of mRNA derived from RCC cell lines (mAbG250$^+$ and mAbG250-), surgical specimens (RCC and normal kidney obtained from the same patient), and normal human organs, revealed a single 1.6 kb transcript in mAbG250$^+$ cell lines and RCC specimens. No transcript was observed in mAbG250 cell lines, normal kidney specimens, nor any normal human organ investigated. Sequence analysis and database searching revealed that the G250 antigen is homologous with MN, a recently cloned human tumor-associated protein of 54/58 kD. Screening of a Lambda-FIX library resulted in the identification of six clones (EO1-6) containing different inserts, spanning the entire G250 gene. Preliminary evidence indicates that the G250 gene is located on chromosome 9. Further molecular characterization of this RCC-associated antigen may result in a better understanding of the molecular basis of kidney carcinogenesis.

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IDENTIFICATION OF DD3: A NEW GENE OVEREXPRESSED IN PROSTATIC TUMORS. *Marion HI Bussemakers, Adrie van Bokhoven, Maud Elfring, Marcel JW Janssen, Ning Ru*, William B Isaacs*.* Urology Research Laboratory, University Hospital Nijmegen, The Netherlands, *Brady Urological Institute, Johns Hopkins Hospital, Baltimore, USA

Identification of genes specifically expressed in tumor cells but not in normal cells, or *vice versa*, is important for the understanding of the molecular basis of carcinogenesis. Furthermore, such genes may provide us with markers for early tumor detection. Recently, advanced molecular genetic tools like comparative genomic hybridization (CGH) revealed that besides frequent loss also amplification of chromosomal loci occurs in prostatic tumors.

Amplification of chromosomal regions is often associated with activation of transcription units (e.g. overexpression). Therefore, we applied the technique of differential display analysis to identify genes overexpressed in prostatic tumors; this technique has been proven to be a powerful tool to identify and clone differentially expressed genes. In our study, mRNA from normal, benign hyperplastic and tumor prostatic tissue from the same patients was extracted and used for differential display. We identified several apparently differentially expressed mRNAs, one of them (DD3) detecting two transcripts (2.2 and 4.0 kb) that are specifically expressed in human prostatic tumors whereas no expression of these transcripts was found in normal or benign hyperplastic prostate tissue. Also in normal human lung, bladder, colon, pancreas, skin, heart, smooth muscle and kidney tissue, no DD3 transcripts could be detected.

Nucleotide sequence analysis of DD3 (1.6 kb) did not reveal an open reading frame nor did we find homology with any known gene. Isolation of additional DD3-related cDNA clones allowed a further characterization of the transcription unit of DD3 and showed that alternative / cryptic splicing occurs, giving rise to the differently sized transcripts.

To assess whether DD3 may be located in an amplified chromosomal region, we isolated DD3-related genomic clones which were used as probes to hybridize to metaphase chromosomes of lymphocytes. In this way we were able to map DD3 to chromosome 9q21-22, a region which has been shown to be amplified in a number of prostatic tumors, suggesting that overexpression of the gene may be a result of gene amplification.

In conclusion, by using differential display we have identified a new gene, DD3 which is overexpressed in prostatic tumors. Further studies will be required to elucidate the function of DD3, its putative role in prostate tumorigenesis and its potential usefulness in prostate cancer diagnosis/prognosis.